PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Preben LEXOW

Appln. No.: **Continuation of PCT/GB99/04417** Group Art Unit: **0000**

Filed: **June 22, 2001** Examiner: **Unknown**

For: **SEQUENCING METHOD USING MAGNIFYING TAGS**

PRELIMINARY AMENDMENT

Assistant Commissioner
of Patents
Washington, D.C. 20231

Sir:

Prior to examining the above-identified application, please amend the application as follows.

IN THE SPECIFICATION:

Please amend the specification as follows:

Page 1, before line 3, insert

-- This Application is a Continuation of PCT/GB99/04417 (published under PCT Article 21(2) in English) filed December 23, 1999. The disclosure of PCT/GB99/04417 is incorporated herein by reference. --

Page 9, lines 27-37 and Page 10, lines 1-5, delete in their entirety, and insert therefor

-- In an exemplary procedure, all target molecules that do not end with AAAA (SEQ ID NO:1) (when the probe ends in TTTT (SEQ ID NO:2)) would not bind and would be removed. Similarly at other addresses, target molecules having particular end sequences would bind selectively. The target molecules may be double

stranded (with single stranded overhangs) or single stranded such that sequences could be bound to and identified at the terminal ends or also internally, respectively. If PNA was used as the complementary probe, since such molecules are able to bind to double stranded forms, internal sequences of double stranded forms could also be bound. In general terms this technique is referred to herein as sorting based on one or several end base pairs and may be performed in one or more cycles. This technique may be coupled to other techniques as described herein. --.

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-- In general terms an example of the process may be described as follows. Base pairs in the target nucleic acid material are associated with four different tags (hereafter called magnifying tags) that represent each of the four bases Adenine, Cytosine, Guanine, and Thymine. Thus, where there was an A-T base pair "magnifying tag A" is associated, C-G is associated with "magnifying tag C", etc. Thereby new DNA molecules are generated where the original base order of e.g. ACGTT (SEQ ID NO:3) is augmented by "magnifying tag A" - "magnifying tag C" - "magnifying tag G", etc. Each magnifying tag provides a means of producing a signal and may in a preferred feature be a polynucleotide molecule. In that case the length of the four tags may vary from two base pairs to several hundred kbp (or more if desired), according to requirements. Correspondingly, the DNA fragments can contain reporter genes and other

biological information or consist only of sequences without a known biological function. --.

Page 20, lines 33-37 and Page 21, lines 1-7, delete in their entirety, and insert therefor

-- Appropriate adapters may then be used to bind to, and thereby allow magnification of, one or more bases of the overhang. In the case of single base magnification, degenerate adapters having single stranded portions of the form, e.g. for a four base overhang, ANNN (SEQ ID NO:4), TNNN (SEQ ID NO:5), CNNN (SEQ ID NO:6) and GNNN (SEQ ID NO:7) and magnifying tags A, T, C and G, respectively may be used. Alternatively the adapters may carry more than one magnifying tags corresponding to more than one of the overhang bases, e.g. having an overhang of ATGC (SEQ ID NO:17), with corresponding magnifying tags to one or more of those bases attached in linear fashion where appropriate. --.

Page 34, lines 5-19, delete in their entirety, and insert therefor

-- The DNA molecules in well 1 have AAAA (SEQ ID NO:1) overhangs, while the DNA molecules in well 2 have AAAC (SEQ ID NO:8) overhangs, etc. The 256 wells thereby cover all permutations of overhangs on four bases. When the target DNA are added to the wells together with ligase, the DNA molecules with TTTT (SEQ ID NO:2) overhangs will attach themselves to well 1, the target DNA with TTTG (SEQ ID NO:9) overhangs to well 2, etc. After having washed off target DNA molecules that were not ligated to the sorting adapters, IIS enzyme is added so that the target DNA molecules are freed at the same time as a new overhang is created that represents the next four base pairs

in the target sequence. This overhang can then be used as the starting point for a new round of sorting, or one may proceed with conversion/magnification. --;

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-- Instead of using different wells, an alternative would be to use different positions on a "microarray". At address 1 it is only DNA molecules that end with TTTT (SEQ ID NO:2) that are fixed, at address 2 it is DNA molecules with TTTG (SEQ ID NO:9) ends that are fixed, etc. Other alternatives are to let DNA molecules with different ends attach/convert at different times, the use of gel sorting, etc. --; and

Page 34, lines 34-37 and Page 35, lines 1-11, delete in their entirety, and insert therefor

-- For example, one may use a strategy where there are 256 different sorting adapters distributed among 256 squares on a "microarray". In square 1, there are sorting adapters with AAAA (SEQ ID NO:1) overhangs, in square 2, they have AAAC (SEQ ID NO:8) overhangs, etc. Thus, the target DNA molecules will be sorted so that those with TTTT (SEQ ID NO:2) overhangs are attached to square 1, GTTT (SEQ ID NO:10) overhangs to square 2, etc. By also fixing the other end of the DNA piece to the substrate, e.g. with biotin/streptavidin, one can then continue to the next conversion/magnification step without the DNA molecules leaving their position on the reading plate. Another strategy for preventing the DNA molecules from leaving their positions is to use a reading plate that is divided into 256 wells/spaces. --.

Page 56, lines 25-31, delete in their entirety, and insert therefor

-- If sorting is used, it is possible to sort the same piece of sequence several times. For example, all target DNA that begin with AAAA (SEQ ID NO:1) are sorted into well 1. Then the same procedure is repeated where incorrectly sorted DNA molecules that do not end with AAAA (SEQ ID NO:1) are washed away. The procedure can in principle be repeated until the desired error percentage is obtained. --.

Page 61, lines 19-24, delete in their entirety, and insert therefor

-- Figure 24 shows the sorting method described herein which is performed on a microarray in which overhangs of 4 bases in the target DNA are mixed with a microarray with 256 addresses and ligated. Address 1 contain AAAA (SEQ ID NO:1) overhangs and thus binds to target molecules with TTTT (SEQ ID NO:2) overhangs; and --.

Page 62, lines 11-25, delete in their entirety, and insert therefor

-- 2. Base pairs in the DNA pieces are replaced with four different DNA sequences (hereafter called DNA fragments, corresponding to the magnifying tags) that represent each of the four bases Adenine, Cytosine, Guanine, and Thymine. Thus, where there was an A-T base pair "fragment A" is inserted, C-G is replaced by "fragment C", etc. Thereby new DNA molecules are generated where the original base order of e.g. ACGTT (SEQ ID NO:3) is replaced by fragment A - fragment C - fragment G, etc. The length of these four DNA fragments can, in principle, vary

from two base pairs to several hundred kbp (or more if desired), according to requirements. Correspondingly, the DNA fragments can contain reporter genes and other biological information or consist only of sequences without a known biological function. --.

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-- Since the overhangs with target DNA in the above-mentioned example seek complementary overhang, each converted DNA piece will be hybridized/ligated to encountered DNA pieces. This creates a chain of magnifying tags (signal chain) that provides information about sequence pieces of 8 base pairs interrupted by 22 unknown bases (e.g. AGCTGTGA N22 AGTCTGCA N22 TGAC (SEQ ID NO:11)). The number of unknown base pairs is determined by the initial length of the DNA piece minus the number of base pairs converted per DNA piece. Based on overlaps between signal chains, it is then possible to reconstruct the target sequence even in areas with repetitive sequences. --.

Page 80, lines 31-37 and Page 81, line 1, delete in their entirety, and insert therefor

-- Methods

1) The starting point is a scanning surface consisting of 65,536 addresses. A perpendicular anchoring line with single-stranded octamers is attached to each address. AAAAAAAA (SEQ ID NO:12) octamers are anchored to the plate at address 1, AAAAAAAC (SEQ ID NO:13) octamers to the plate at address 2, etc, so that all the 65,536 octamer permutations each have their own address. --.

Page 83, lines 10-20, delete in their entirety, and insert therefor

-- 7) The target DNA is distributed from the 256 wells between 256 microarrays as described in Example 12. All the microarrays are alike and consist of 256 addresses with sorting adaptors with 4-base overhangs that can complement the overhangs made in step 6). At address 1 the sorting adaptors have AAAA (SEQ ID NO:1) overhangs, at address 2 they have AAAC (SEQ ID NO:8) overhangs etc.

8) Ligase is added and the mixture incubated. At address 1 there will be target DNA with TTTT (SEQ ID NO:2) overhangs, at address 2 there will be target DNA with TTTG (SEQ ID NO:9) overhangs, etc. --.

Page 84, lines 14-26, delete in their entirety, and insert therefor

-- Results

The presence or absence and size of molecules at particular addresses indicates both sequence information and its position. Thus if address 1 of microarray 1 contains DNA molecules of 100 micrometers, this indicates that the sequence corresponding to the octamer used (albeit by 2-step sorting) to bind that molecule is present at +200kb (e.g. TTTTTTTT (SEQ ID NO:14)). Similar the presence of 2 differently sized molecules would indicate a repeat of the particular sequence. The absence of any molecules at a particular address would indicate the absence of the sequence complementary to the immobilizing octamer in the target sequence. --.

Page 94, lines 26-30, delete in their entirety and insert therefor

-- Method

1) The DNA molecule that is to be sequenced, ACGTGAGCT (SEQ ID NO:15) is fixed with one end to a streptavidin-covered plate. The fixing mechanism should be a mechanism other than streptavidin/biotin. --

Page 95, lines 1-6, delete in their entirety, and insert therefor

-- 4) A solution with various adapters and ligases is then added. Figure 19 shows an adapter that has recognized and bound to the ACGT (SEQ ID NO:16) overhang. In addition to fragments labelled with fluorescence that correspond to the ACGT (SEQ ID NO:16) overhang, two or more biotin molecules have been incorporated on the adapter. --

Page 96, lines 36-37 and Page 97, lines 1-8, delete in their entirety, and insert therefor

-- Method

A DNA chip is used which is divided into 256 addresses. Each address contains sorting adaptors, an overhang, a binding site for a class IIS restriction endonuclease and a binding site for a restriction endonuclease that makes a blunt end cut. The overhangs vary from address to address so that address 1 has sorting adaptors with an AAAA (SEQ ID NO:1) overhang, address 2 has an AAAC (SEQ ID NO:8) overhang, etc. In addition all the addresses are covered with a molecule with binding properties, e.g. streptavidin. --.

Page 97, lines 13-18, delete in their entirety, and insert therefor

-- 2) The fragments are introduced to the solid support carrying the sorting adaptors to which they are ligated. DNA pieces with a TTTT (SEQ ID NO:2) overhang will ligate to address 1 in which the sorting adaptors have the complementary overhang AAAA (SEQ ID NO:1), DNA pieces with a GTTT (SEQ ID NO:10) overhang will ligate to address 2 etc. --.

IN THE CLAIMS:

Please cancel Claims 1-25.

Please add the following new claims:

-- Claim 26. A method of magnifying a signal associated with one or more bases of a sequence in a target nucleic acid molecule comprising the steps of:

(A) treating said target nucleic acid molecule so that at least a region of said target nucleic acid molecule is converted into a form suitable for binding an adapter molecule (adapter binding region);

wherein said adapter molecule comprises:

(i) one or more of said magnifying tags, or

(ii) a means for attaching one or more of said magnifying tags,

wherein said magnifying tag(s) correspond to one or more bases of the adapter binding region or to one or more bases in proximity to the adapter binding region;

(B) binding said adapter molecule to at least a portion of said adapter binding region created in step (A) to form a nucleic acid molecule:adapter molecule complex;

(C) optionally, ligating said target nucleic acid molecule to said adapter molecule such that at least said magnifying tag(s) remain associated with said target nucleic acid molecule;

(D) treating the resulting complex of step (B) or step (C) so that at least another region of said target nucleic acid molecule is converted into a form suitable for binding another adapter molecule, wherein said another region comprises one or more bases which are not associated with the magnifying tags of step (B); and thereafter

(E) repeating steps (B) to (D), with the proviso that the adapter molecule in each cycle of steps (B) to (C) binds to a region adjacent to a region of said target nucleic acid molecule to which the adapter molecule of a previous cycle bound, or the adapter molecule in each cycle of steps (B) to (C) binds to a region which overlaps with a region of said target nucleic acid molecule to which the adapter molecule of a previous cycle bound, and wherein the magnifying tags of each cycle of steps (A) to (C) are ligated together,

to thereby magnify said signal.

Claim 27. The method as claimed in Claim 26, wherein in step (A), said form is a single-stranded nucleic acid molecule.

Claim 28. The method as claimed in Claim 26, wherein said magnifying tag(s) correspond to one or more bases of said adapter binding region.

Claim 29. The method as claimed in Claim 26, wherein each magnifying tag corresponds to at least 2 bases in said adapter binding region or to at least 2 bases adjacent to said adapter binding region.

Claim 30. The method as claimed in Claim 26, wherein said magnifying tags together correspond to at least 2 bases in said adapter binding region or to at least 2 bases adjacent to said adapter binding region.

Claim 31. The method as claimed in Claim 30, wherein said magnifying tags together correspond to at least 4 bases in said adapter binding region or to at least 4 bases adjacent to said adapter binding region.

Claim 32. The method as claimed in Claim 26, wherein a chain of magnifying tags are associated with said target nucleic acid molecule.

Claim 33. The method as claimed in Claim 32, wherein said chain comprises 4 or more magnifying tags corresponding to at least 4 contiguous bases.

Claim 34. The method as claimed in Claim 26, wherein said magnifying tags are nucleic acid sequences of at least 2 bases in length.

Claim 35. The method as claimed in Claim 34, wherein said magnifying tags are nucleic acid sequences of 10 to 30 bases in length.

Claim 36. The method as claimed in Claim 26, wherein said adapter molecule comprises a recognition site for a nuclease, which has a cleavage site separate from its recognition site.

Claim 37. The method as claimed in Claim 26, wherein said adapter molecule comprises recognition sites for 2 or more nucleases, which have cleavage sites separate from their respective recognition sites, wherein cleavage with said nucleases produces single-stranded regions which are adjacent or overlapping.

Claim 38. The method as claimed in Claim 26, wherein two or more adapter molecules are bound in step (B).

Claim 39. The method as claimed in Claim 38, wherein said adapter molecules are bound to overlapping or adjacent regions.

Claim 40. The method as claimed in Claim 39, wherein said adapter molecules are bound to overlapping regions thereby allowing the association of more than one magnifying tag with each base.

Claim 41. The method as claimed in Claim 26, wherein step (C) is performed.

Claim 42. The method as claimed in Claim 26, further comprising the step of:

(F) sequencing the target nucleic acid molecule by identifying the magnifying tags associated with the target nucleic acid molecule.

Claim 43. The method as claimed in Claim 42, wherein 2 or more bases are sequenced per cycle.

Claim 44. The method as claimed in Claim 43, wherein 4 or more bases are sequenced per cycle.

Claim 45. The method as claimed in Claim 42, wherein the signal associated with each base is magnified by increasing the number of times that said base appears in said sequence.

Claim 46. The method as claimed in Claim 42, wherein the resulting magnified signal is converted into readable signals and said sequencing is carried out by assessing the readable signals.

Claim 47. The method as claimed in Claim 46, wherein each readable signal comprises a pattern made up of a single signal event which creates a unique signal on each magnifying tag.

Claim 48. A method of sequencing all or part of a target nucleic acid molecule comprising the steps of:

(A) determining the sequence of a portion of said target nucleic acid molecule;

(B) determining the position of said portion within said target nucleic acid molecule; and

(C) combining the information obtained in steps (A) and (B) to obtain the sequence of all or part of said target nucleic acid molecule.

Claim 49. The method as claimed in Claim 48, wherein said position is determined by reference to a positional marker.

Claim 50. The method as claimed in Claim 48, wherein said position is determined by reference to a restriction map of said target nucleic acid molecule.

Claim 51. The method as claimed in Claim 48, wherein the portion which is sequenced has 4 or more bases and/or the position of said portion within said target nucleic acid molecule is determined with an accuracy of less than 1 kb.

Claim 52. The method as claimed in Claim 48, wherein said portion is sequenced by identifying magnifying tags associated with the target nucleic acid molecule, wherein said magnifying tags correspond to one or more bases of an adapter binding region or to one or more bases in proximity to an adapter binding region, wherein said adapter binding region binds an adapter molecule which comprises:

(i) one or more of said magnifying tags, or

(ii) a means for attaching one or more of said magnifying tags.

Claim 53. The method as claimed in Claim 48, wherein the sequence of the target nucleic acid molecule is determined by assessing the complementarity of a portion of said target nucleic acid molecule by a process comprising the steps of:

(i) treating said target nucleic acid molecule so that at least a region of said target nucleic acid molecule is converted into a form suitable for binding a complementary probe, wherein said complementary probe is bound to a solid support or said complementary probe carries a means for attaching to a solid support;

(ii) binding said complementary probe to at least a portion of said region suitable for binding a complementary probe;

(iii) optionally repeating steps (i) and (ii), with the *proviso* that said complementary probe binds to an adjacent or overlapping region of said target nucleic acid molecule relative to the region to which the complementary probe of the previous cycle bound; and

(iv) determining the sequence of said target nucleic acid molecule by identifying the complementary probe(s) to which said target nucleic acid molecule bound.

Claim 54. The method as claimed in Claim 53, wherein in step (i) said form is a single-stranded nucleic acid molecule.

Claim 55. The method as claimed in Claim 53, wherein in step (ii) said portion is 4 to 12 bases in length.

Claim 56. The method of as claimed in Claim 43, wherein a portion of said sequence is determined by identifying magnifying tags associated with the target nucleic acid molecule, wherein said magnifying tags correspond to one or more bases of an adapter binding region or to one or more bases in proximity to an adapter binding region, wherein said adapter binding region binds an adapter molecule which comprises:

(i) one or more of said magnifying tags, or

(ii) a means for attaching one or more of said magnifying tags; and

an adjacent or overlapping portion of said sequence is determined by a process comprising the steps of:

(i) treating said target nucleic acid molecule so that a region of said target nucleic acid

molecule is converted into a form suitable for binding a complementary probe, wherein said complementary probe is bound to a solid support or said complementary probe carries a means for attaching to a solid support;

(ii) binding said complementary probe to at least a portion of said region suitable for binding a complementary probe;

(iii) optionally repeating steps (i) and (ii), with the *proviso* that said complementary probe binds to an adjacent or overlapping region of said target nucleic acid molecule relative to the region to which the complementary probe of the previous cycle bound; and

(iv) determining the sequence of said target nucleic acid molecule by identifying the complementary probe(s) to which said target nucleic acid molecule bound.

Claim 57. A kit for magnifying one or more bases of a target nucleic acid molecule comprising at least one or more adapter molecules as defined in Claim 26.

Claim 58. The kit as claimed in Claim 57, wherein said adapter molecules are attached to one or more solid supports.

Claim 59. The method as claimed in Claim 26, wherein said method is performed on a sample comprising a heterogeneous mixture of target nucleic acid molecules.

Claim 60. The method as claimed in Claim 42, wherein said method is performed on a sample comprising a heterogeneous mixture of target nucleic acid molecules.

Claim 61. The method as claimed in Claim 48, wherein said method is performed on a sample comprising a heterogeneous mixture of target nucleic acid molecules.

Claim 62. A method of producing a map of a target nucleic acid molecule comprising the steps of:

(A) obtaining sequence information on portions of a target nucleic acid molecule by cleaving said target nucleic acid molecule with one or more nucleases; and

(B) binding an adapter molecule to a region of said target nucleic acid molecule, wherein said adapter molecule comprises one or more magnifying tags as claimed in Claim 26,

wherein each tag comprises:

(i) a first signaling moiety which corresponds to one or more bases of said region to which said adapter molecule binds, and

(ii) a second signaling moiety which corresponds to a nuclease used for cleavage,

wherein said portions comprise all or part of the cleavage sites of said nucleases and/or all or part of the restriction sites of said nucleases; and

(C) determining the position of said portions within said target nucleic acid molecule so to produce a map of the target nucleic acid molecule.

Claim 63. The method as claimed in Claim 62, wherein said nuclease has a cleavage site which is separate from its recognition site.

Claim 64. The method as claimed in Claim 62, wherein said cleaving produces complementary single-stranded regions. --

IN THE ABSTRACT:

Please insert the Abstract attached hereto.

SEQUENCE LISTING:

Please insert the Sequence Listing filed simultaneously herewith.

REMARKS

The specification has been amended to insert formal matter; Claim 1-25 have been cancelled and new Claims 26-60 are being amended in order to remove improper dependency and conform with U.S. patent practice; and the Abstract and Sequence Listing has been added in order to make the application consistent with U.S. patent practice.

The Examiner is requested to note that Applicant simultaneously files herewith a Sequence Listing (which is considered to be a separate document by the U.S. Patent and Trademark Office) in PatentIn Version 3.0.

Hence, the amendments to the specification and claims, and the addition of the Abstract and Sequence Listing do not constitute new matter.

The Examiner is invited to contact the undersigned at his Washington telephone number on any questions which might arise.

Respectfully submitted,

Gordon Kit
Registration No. 30,764

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Date: June 22, 2001

A P P E N D I X

Marked-Up Version of Amendments

IN THE SPECIFICATION:

The specification is amended as follows:

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In an exemplary procedure, all target molecules that do not end with AAAA (SEQ ID NO:1) (when the probe ends in TTTT (SEQ ID NO:2)) would not bind and would be removed. Similarly at other addresses, target molecules having particular end sequences would bind selectively. The target molecules may be double stranded (with single stranded overhangs) or single stranded such that sequences could be bound to and identified at the terminal ends or also internally, respectively. If PNA was used as the complementary probe, since such molecules are able to bind to double stranded forms, internal sequences of double stranded forms could also be bound. In general terms this technique is referred to herein as sorting based on one or several end base pairs and may be performed in one or more cycles. This technique may be coupled to other techniques as described herein.

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Appropriate adapters may then be used to bind to, and thereby allow magnification of, one or more bases of the overhang. In the case of single base magnification, degenerate adapters having single stranded portions of the form, e.g. for a four base overhang, ANNN (SEQ ID NO:4), TNNN (SEQ ID NO:5), CNNN (SEQ ID NO:6) and GNNN (SEQ ID NO:7) and magnifying tags A, T, C and G, respectively may be used. Alternatively the adapters may carry more than one magnifying tags corresponding to more than one of the overhang bases, e.g. having an overhang of ATGC (SEQ ID NO:17), with corresponding magnifying tags to one or more of those bases attached in linear fashion where appropriate.

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sorted so that those with TTTT (SEQ ID NO:2) overhangs are attached to square 1, GTTT (SEQ ID NO:10) overhangs to square 2, etc. By also fixing the other end of the DNA piece to the substrate, e.g. with biotin/streptavidin, one can then continue to the next conversion/magnification step without the DNA molecules leaving their position on the reading plate. Another strategy for preventing the DNA molecules from leaving their positions is to use a reading plate that is divided into 256 wells/spaces.

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Figure 24 shows the sorting method described herein which is performed on a microarray in which overhangs of 4 bases in the target DNA are mixed with a microarray with 256 addresses and ligated. Address 1 contain AAAA (SEQ ID NO:1) overhangs and thus binds to target molecules with TTTT (SEQ ID NO:2) overhangs; and

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replaced by "fragment C", etc. Thereby new DNA molecules are generated where the original base order of e.g. ACGTT (SEQ ID NO:3) is replaced by fragment A - fragment C - fragment G, etc. The length of these four DNA fragments can, in principle, vary from two base pairs to several hundred kbp (or more if desired), according to requirements. Correspondingly, the DNA fragments can contain reporter genes and other biological information or consist only of sequences without a known biological function.

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Since the overhangs with target DNA in the above-mentioned example seek complementary overhang, each converted DNA piece will be hybridized/ligated to encountered DNA pieces. This creates a chain of magnifying tags (signal chain) that provides information about sequence pieces of 8 base pairs interrupted by 22 unknown bases (e.g. AGCTGTGA N22 AGTCTGCA N22 TGAC (SEQ ID NO:11)). The number of unknown base pairs is determined by the initial length of the DNA piece minus the number of base pairs converted per DNA piece. Based on overlaps between signal chains, it is then possible to reconstruct the target sequence even in areas with repetitive sequences.

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Methods

1) The starting point is a scanning surface consisting of 65,536 addresses. A perpendicular anchoring line with single-stranded octamers is attached to each address. AAAAAAAA (SEQ ID NO:12) octamers are anchored to the plate at address 1, AAAAAAAC (SEQ ID NO:13) octamers to the plate at

[address 2,etc,] address 2, etc, so that all the 65,536 octamer permutations each have their own address.

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7) The target DNA is distributed from the 256 wells between 256 microarrays as described in Example 12. All the microarrays are alike and consist of 256 addresses with sorting adaptors with 4-base overhangs that can complement the overhangs made in step 6). At address 1 the sorting adaptors have AAAA (SEQ ID NO:1) overhangs, at address 2 they have AAAC (SEQ ID NO:8) overhangs etc.

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Results

The presence or absence and size of molecules at particular addresses indicates both sequence information and its position. Thus if address 1 of microarray 1 contains DNA molecules of [100micrometers] 100 micrometers, this indicates that the sequence corresponding to the octamer used (albeit by 2-step sorting) to bind that molecule is present at +200kb (e.g. TTTTTTTT (SEQ ID NO:14)). Similar the presence of 2 differently sized molecules would indicate a repeat of the particular sequence. The absence of any molecules at a particular address would indicate the absence of the sequence complementary to the immobilizing octamer in the target sequence.

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Page 95, lines 1-6 are changed as follows:

4) A solution with various adapters and ligases is then added. Figure 19 shows an adapter that has recognized and bound to the ACGT (SEQ ID NO:16) overhang. In addition to fragments labelled with fluorescence that correspond to the ACGT (SEQ ID NO:16) overhang, two or more biotin molecules have been incorporated on the adapter.

Page 96, lines 36-37 and Page 97, lines 1-8 are changed as follows:

Method

A DNA chip is used which is divided into 256 addresses. Each address contains sorting adaptors, an overhang, a binding site for a class IIS restriction endonuclease and a binding site for a restriction endonuclease that makes a blunt end cut. The overhangs vary from address to address so that address 1 has sorting adaptors with an AAAA (SEQ ID NO:1) overhang, address 2 has an AAAC (SEQ ID NO:8) overhang, etc. In addition all the addresses are covered with a molecule with binding properties, e.g. streptavidin.

Page 97, lines 13-18 are changed as follows:

2) The fragments are introduced to the solid support carrying the sorting adaptors to which they are ligated. DNA pieces with a TTTT (SEQ ID NO:2) overhang will ligate to address 1 in which the sorting adaptors have the complementary overhang AAAA (SEQ

ID NO:1), DNA pieces with a GTTT (SEQ ID NO:10) overhang will ligate to address 2 etc.

IN THE CLAIMS:

Claim 1-25 are being cancelled.

New Claims 26-64 are being added.

IN THE ABSTRACT:

An Abstract is added.

SEQUENCE LISTING:

A Sequence Listing is added.